DNA CALIBRATION OF AB 7500 FOR PLEXOR HY

A. SCOPE

The AB 7500 must be calibrated for fluorescein, CAL Fluor, Orange 560, CAL Fluor Red 610 and IC5 at least semi-annually. This will typically occur after annual maintenance or repair of the instrument, approximately six months after annual maintenance or in other instances when deemed appropriate before the Plexor HY System is utilized. The Plexor Calibration Kit, Set A, includes aliquots of these four calibrators at a 100X concentration, along with a calibration buffer for use as a diluent to create a spectral calibration plate for each dye. Before performing the following dye calibration, perform a background calibration (if it has not already been completed as part of the annual instrument maintenance).

B. QUALITY CONTROL

B.1 Protective gloves, a lab coat, and eye protection (e.g. safety glasses or a face shield) must be worn at all times when performing this procedure.

B.2 Optical plates should be kept in the appropriate base at all times, including plate setup and centrifugation. This limits the amount of debris introduced into the AB 7500 instrument and prevents damage to plate wells that may affect optical reading.

C. SAFETY

C.1 Protective gloves, a lab coat, and eye protection (e.g. safety glasses or a face shield) must be worn at all times when performing this procedure.

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 Plexor Calibration Kit, Set A (Promega)

D.2 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 AB 7500 Real-Time PCR instrument and software
E.1.2 Microcentrifuge
E.1.3 Pipettes
E.1.4 Vortexer
E.1.5 96 well plate centrifuge

E.2 Supplies

E.2.1 Kimwipes
E.2.2 Microcentrifuge tubes
E.2.3 Sterile aerosol resistant pipette tips
E.2.4 Microcentrifuge tube racks
E.2.5 AB optical 96-well plates
E.2.6 AB optical adhesive covers
E.2.7 Adhesive seal applicator
E.2.8 96-well plate base
E.2.9 Disposable gloves
E.2.10 Lab coat
E.2.11 Eye protection (e.g. safety glasses or a face shield)

F. PROCEDURE

F.1 Perform a background calibration if it has not already been completed.

F.2 Thaw the four Concentrated Calibrators (fluorescein, CAL Fluor Orange 560, CAL Fluor Red 610 and IC5) and Calibration Buffer.

F.3 Vortex the Concentrated Calibrators and Calibration Buffer to mix.

F.4 For each spectral calibrator, dilute 20 µl of Concentrated Calibrator in 1,980 µl of Calibration Buffer.

F.5 Vortex the diluted spectral calibrators for 3–5 seconds to mix.

F.6 For each diluted spectral calibrator, dispense 20 µl to all 96 wells of a 96-well optical plate. Record the barcode number or mark the side of the plate skirt to designate the fluorescein, CAL Fluor Orange 560, CAL Fluor Red 610 and IC5 spectral calibration plates.

F.7 Apply a plate seal to each plate. Protect plates from light.

F.8 Centrifuge plates briefly.

F.9 Load one of the dye plates onto the 7500 instrument.

F.10 Open a new plate document: Filer > New.

F.11 Configure the New Document dialog box:

F.11.1 Select Assay > Pure Spectra
F.11.2 Select Container > 96-Well Clear
F.11.3 Select template > Blank Document
F.11.4 In the Operator field, enter you name

F.11.5 In the Comments field, enter any information that you want to attach to the file
F.11.6 Click Finish.

NOTE: It is not necessary to name or save the pure dye plate document. The SDS software automatically saves the pure dye data to a calibration file on the computer hard drive.

F.12 The Pure Spectra Calibration manager is displayed:
F.12.1 In the Dye list field, select the pure dye, i.e. fluorescein as "FL", CAL Fluor Orange as "CO560", CAL Fluor Red as "CR610", and IC5 as "IC5, that corresponds to the dye plate loaded onto the instrument in F.8 to calibrate.
F.12.2 Click Calibrate.
F.12.3 A message will prompt you to replace the dye sds document; click Yes.
F.12.4 If you are prompted to disconnect the active document, click Yes.
F.12.5 A message will prompt you to load the plate; click Yes.

F.13 When the SDS software completes the run:
F.13.1 Click Ok on the "Run completed successfully"
F.13.2 Press the tray to open it
F.13.3 Remove the pure dye plate from the tray
F.13.4 Press the tray to move it into the instrument
F.13.5 Return the pure dye plate to the freezer.

F.14 Repeat the procedure for each dye plate.

F.15 After the instrument is calibrated with all pure dyes, click Finish.

F.16 Select the Results tab, then select the Spectra tab.

F.17 Select all wells of the plate document by clicking the upper-left corner of the plate grid.

F.18 Click the green triangle to extract pure spectra (or select Analysis>Extract Pure Spectra). The SDS software will complete the extraction, then displays a message:
F.18.1 Pure Spectra Extraction Complete- the analysis is successful. Click ok.
F.18.2 Repair Message. Click ok.
F.18.3 Error Message. Click ok, load the plate, then run the pure dye plate again.

F.19 In the pure dye plate document, select the Results tab, then select the Spectra tab.

F.20 Select all wells of the plate document by clicking the upper-left corner of the plate grid.

F.21 Using the spectra for each pure dye, provided by Promega Inc., as seen in Section G, verify that the peak for the spectrum of the pure dye occurs at the correct filter. If the peak for the spectra of a dye occurs in the wrong filter the wrong dye may have been run during calibration. Repeat the procedure.

F.22 Select File>Close. The SDS software displays the plate document for the next pure dye plate. DO NOT CLOSE A PLATE DOCUMENT UNTIL YOU HAVE EXTRACTED IT.

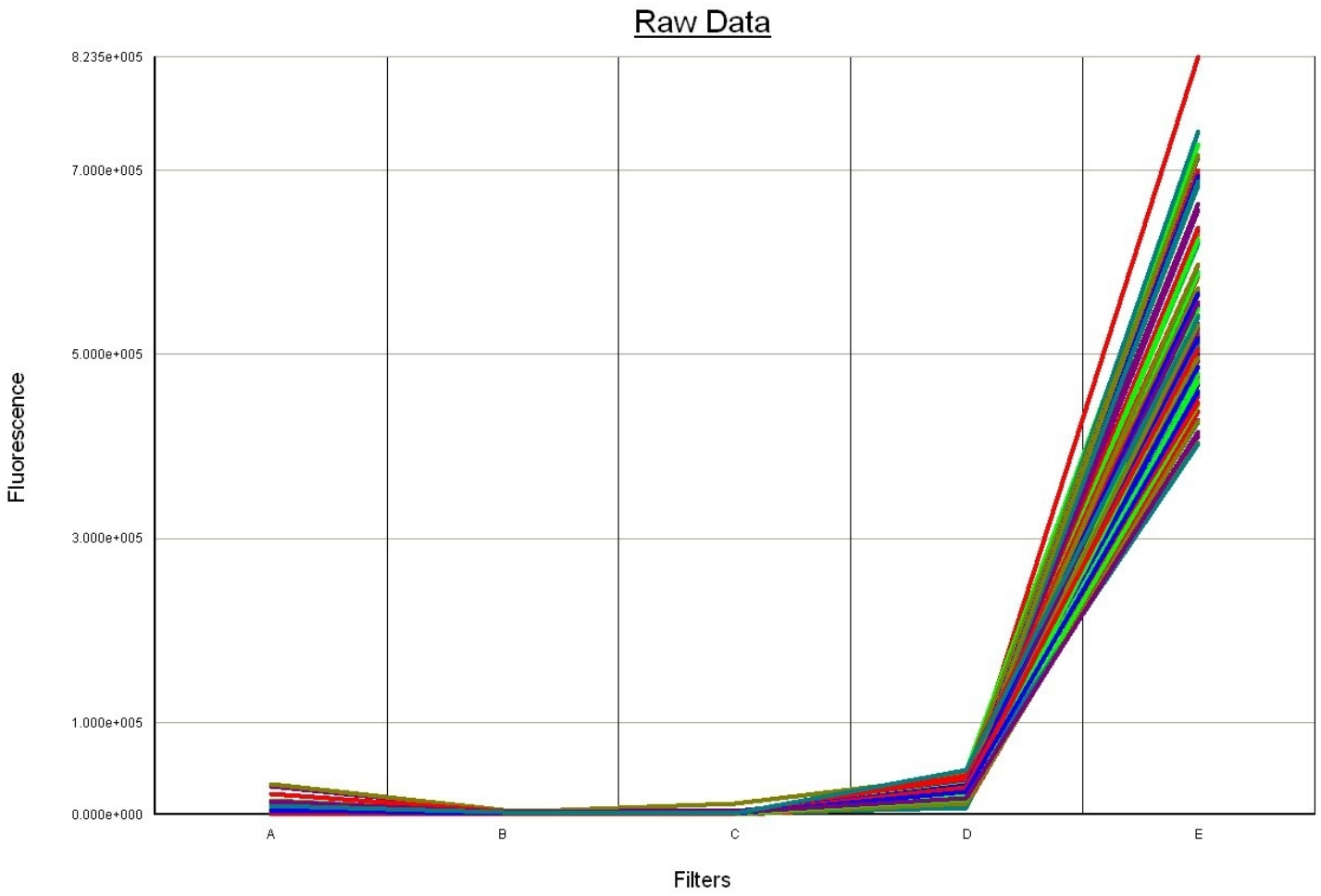
F.23 Repeat the above steps to extract the calibration date for the remaining dyes.

F.24 Once complete, close the remaining plate document.

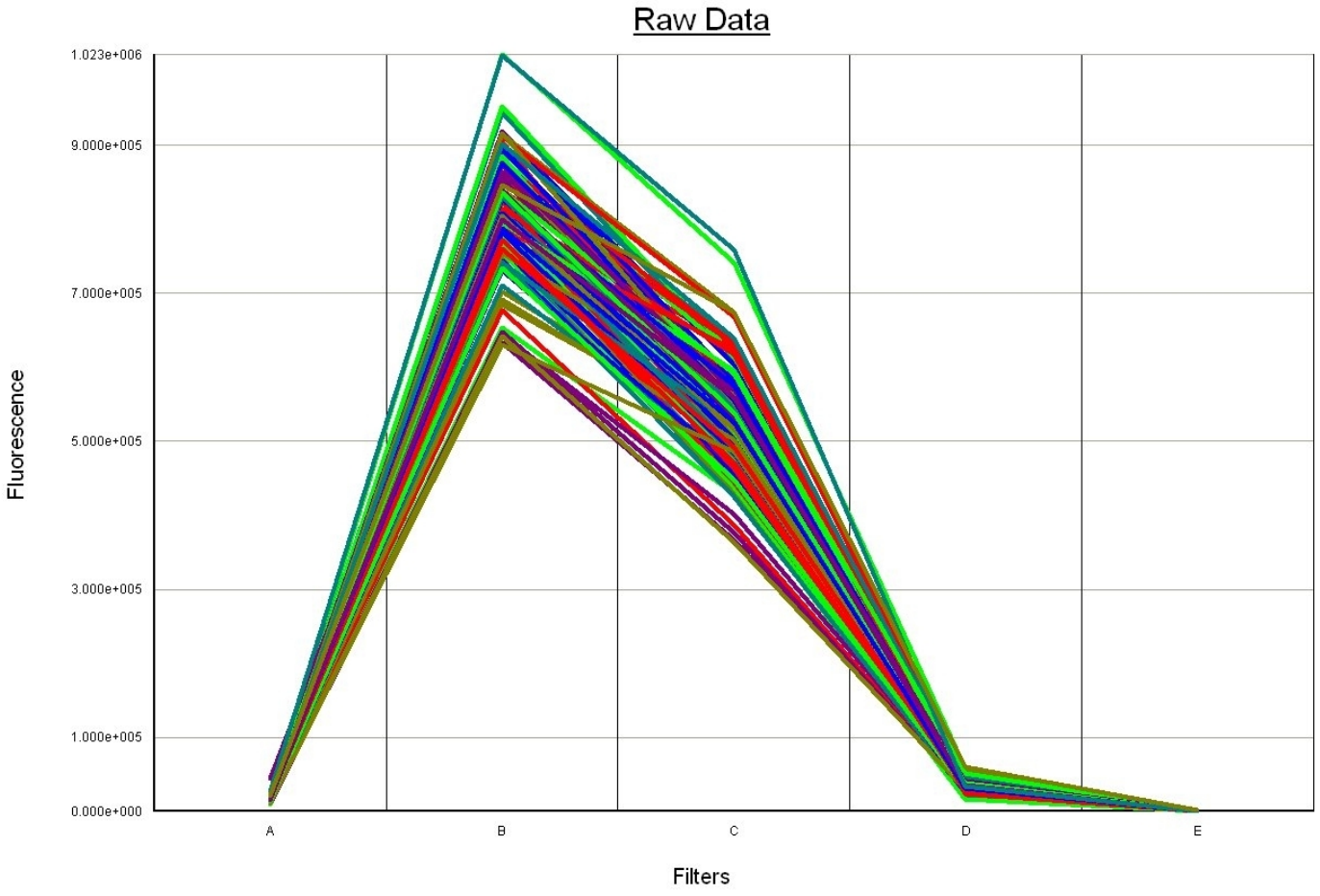
F.25 If the calibration of the 7500 for any of the dyes fail, repeat the calibration with the prepared dye plate. If it continues to fail, prepare a new dye plate and repeat the calibration. If the calibration continues to fail, the instrument will be taken out of service and either Promega or Life Technologies will be contacted.

G. INTERPRETATION GUIDELINES

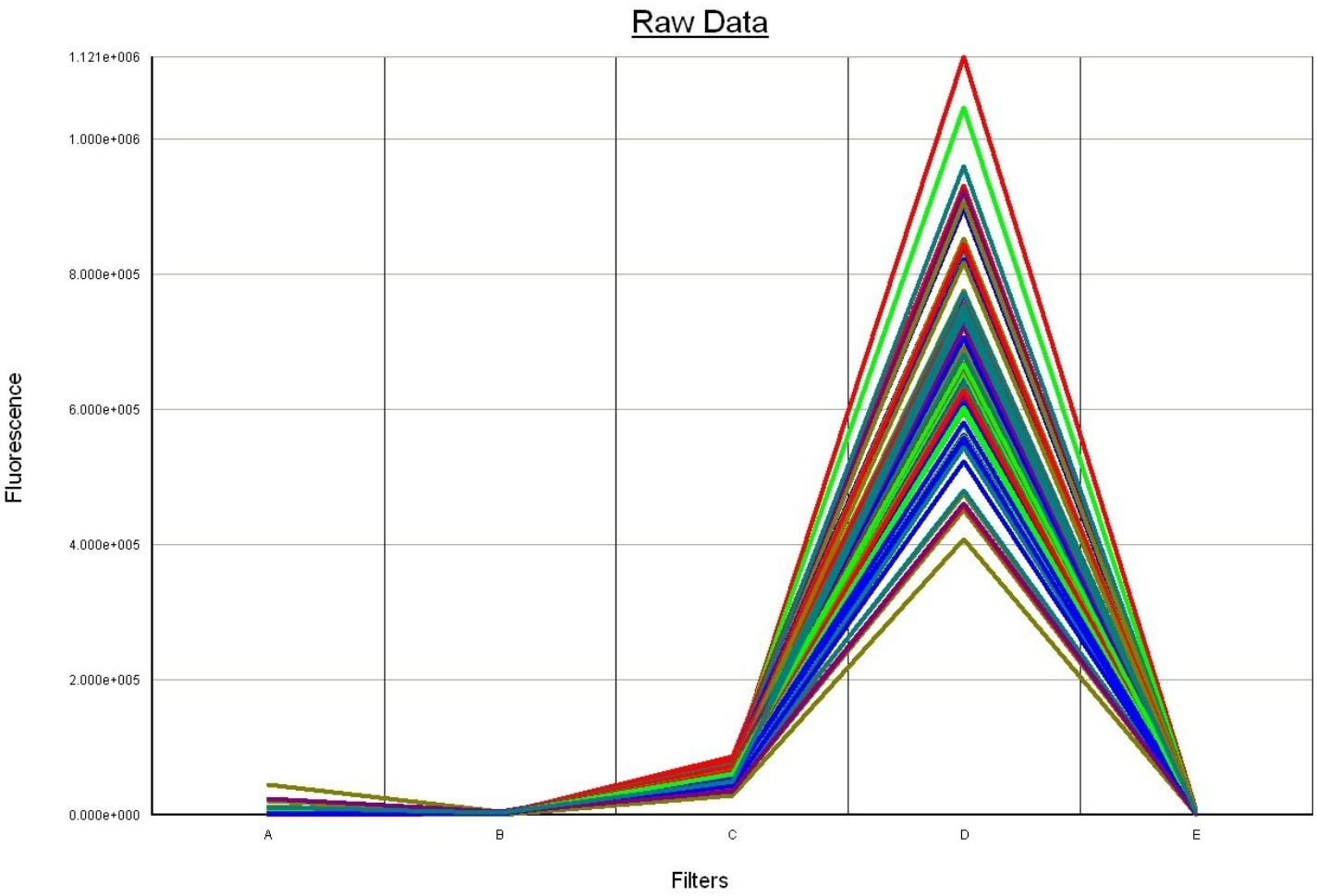
Spectra provided by Promega.



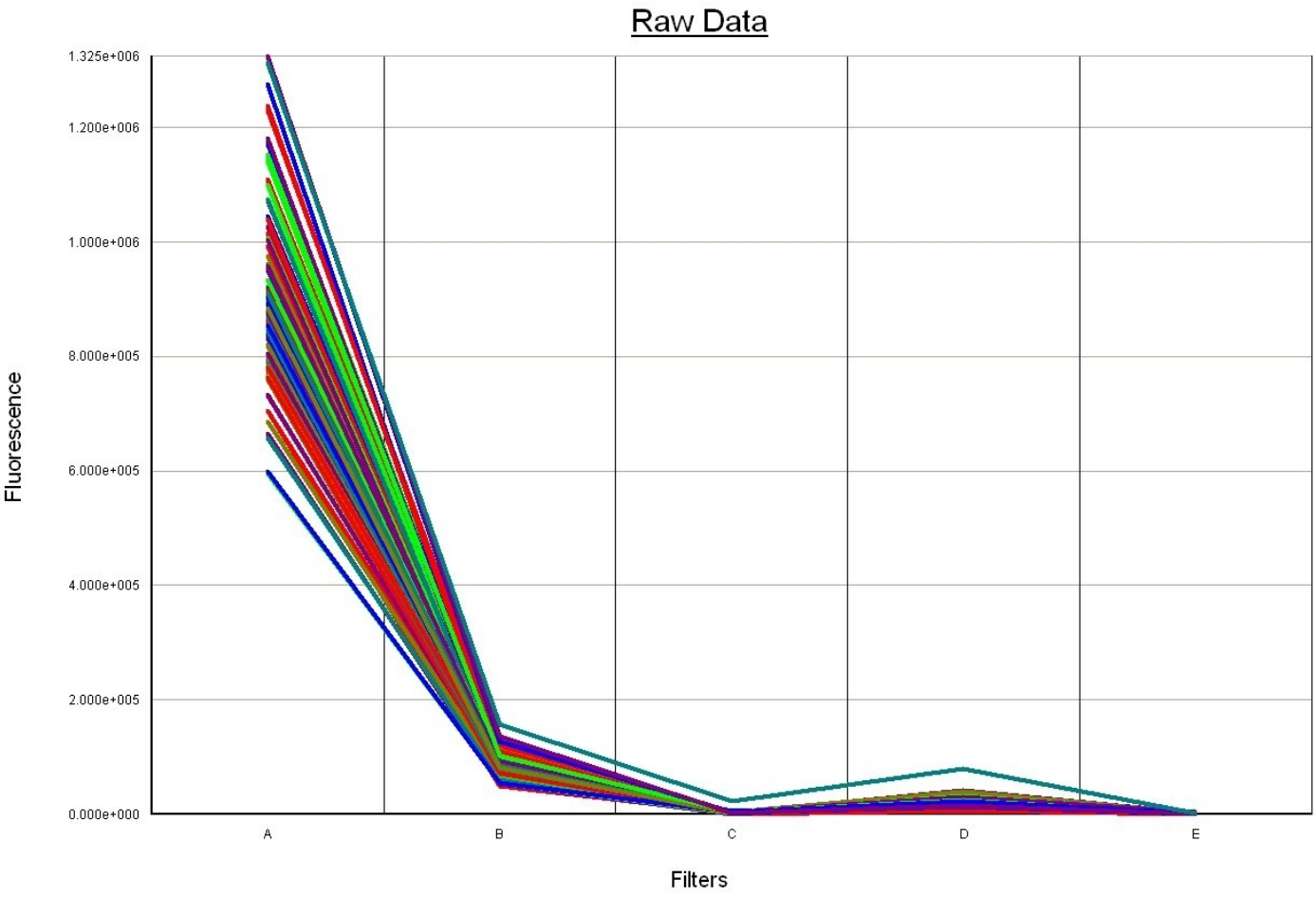
Reading: 1
Well(s): A1-G1,G3-H12
Document: PureSpectra_IC5_Std.sds (Pure Spectra)



Reading: 1
Well(s): A1-H12
Document: PureSpectra_CO560_Std.sds (Pure Spectra)



Reading: 1
Well(s): A1-D4,D6-H12
Document: PureSpectra_CR610_Std.sds (Pure Spectra)



H. REFERENCES

H.1 Plexor HY System for the Applied Biosystems AB 7500 and AB 7500 FAST Real-Time PCR Systems, Instructions for use of products DC1000 and DC1001.

H.2 Applied Biosystems AB 7500/AB 7500 FAST Real-time PCR System; Installation and Maintenance Guide.